SUSCEPTIBILITY TO MAIN ANTIBIOTICS OF GRAM-NEGATIVE BACTERIA FROM BOVINE MASTITIS IN ITALIAN HERDS

Antonio Barberio[1], Clara Locatelli[2], Riccardo Muliari[1], Sandra Bonamico[1], Valerio Bronzo[2], and Paolo Moroni[2,3]
[1]Istituto Zooprofilattico Sperimentale delle Venezie, Sez. Verona-Vicenza, Italy
[2]Università degli Studi di Milano, Milan, Italy
[3]Quality Milk Production Services, Cornell University, Ithaca, New York, USA

Introduction

The incorrect use of antibiotics in veterinary medicine has contributed to reduce the pathogen susceptibility to antibiotics. Through direct contact or food chain the resistant bacteria can be transferred to humans (1). Gram-negative bacteria are frequently the cause of clinical mastitis and, as part of enteric flora, they are subjected to the selective pressure of systemic drug treatments. For this reason they are subjected to specific monitoring program for antibiotic resistance. This paper describes a study about in vitro antimicrobial susceptibility of Gram negative bacteria isolated from bovine clinical mastitis in Northern Italy.

Materials and Methods

Among Gram negative bacteria isolated from bovine clinical mastitis in Northern Italy during the years 2010/2011, 99 isolates were selected: 53 *E.coli*, 28 *Klebsiella* spp, 14 *Serratia* spp, 2 *Enterobacter* spp. e 2 *Citrobacter* spp. Pathogens isolation was carried out in milk samples following the National Mastitis Council guideline (2). Species identification was performed through commercial kits API 20 and API 20 NE (Biomerieux Marcy l'Etoile, France). In vitro antimicrobial susceptibility was evaluated using minimal inhibitory concentrations (MICs) broth dilution test, according to the procedure described in Clinical and Laboratory Standards Institute (CLSI) guideline. CLSI resistance breakpoints were used (3). Sensititre susceptibility plates were used according to supplier instructions (Trek Diagnostics System, East Grinstead, UK). After incubation the plates were manually read recording the last dilution able to inhibit bacterial growth. The antibiotics used are listed in table 1. The antibiotics were selected with the aim to provide epidemiological information on antimicrobial resistance to the different classes of drugs, but we should point out that there are regional differences in approved drugs for treatment of dairy cattle. For example, chloramphenicol, sulfonamides, nitrofurans, and flouroquinolones are prohibited in the US. Aminoglycosides are not recommended due to extended tissue residues.

Results and Discussion

The 36% of tested *E. coli* resulted resistant towards ampicillin, while using penicillins associated to clavulanic acid the percentage of resistant isolates declined to 7,5%. *Serratia* spp isolates demonstrated a higher resistance level to amoxicillin/clavulanic acid, with susceptibility reduced to 74%. Cephalosporins confirmed a very high efficacy in the regards of *E coli*, as 94,3% of isolates were susceptible to 1st generation cephalosporins and 100% to 3rd generation (ceftiofur and cefpodoxime). Similar results were obtained for *Klebsiella* spp, while all *Serratia* spp tested

isolates revealed some resistance to 1st generation cephalosporins, maintaining susceptibility to 3rd generation cephalosporins. Among drugs active specifically towards Gram-negatives bacteria, chloramphenicol and sulfonamide associated with trimethoprim demonstrated high efficacy to all tested species. Average susceptibility was 85% for sulfonamide with trimethoprim, and 95% for chloramphenicol. Finally, only one *E. coli* isolate resulted resistant to fluoroquinolons with an intermediate MIC for marbofloxacin and enrofloxacin, while all the others 98 Gram-negative isolates tested were susceptible (MIC < 0,5 μg/mL). Complete data are shown in table 1.

Table 1. In vitro susceptibility and resistance of *E. coli* and Klebsiella spp., Serratia spp., *Enterobacter* spp. and Citrobacter spp. (joined in "other Gram-negatives") toward tested antibiotics

Antibiotics	Sensible *E. coli* (%)	Resistant *E. coli* (%)	Sensible other Gram-negatives (%)	Resistant other Gram-negatives (%)
Ampicillin	64,2%	35,8%	30,4%	69,6%
Amoxicillin/clavulanic acid	92,5%	7,5%	73,9%	26,1%
Amikacin	100,0%	0,0%	100,0%	0,0%
Cefpodoxime (3rd gen. Ceph.)	100,0%	0,0%	100,0%	0,0%
Ticarcillin/clavulanic acid	94,3%	5,7%	89,1%	10,9%
Sulfonamide/thrimethoprim	86,8%	13,2%	89,1%	10,9%
Cefazolin (1°st gen. Ceph.)	94,3%	5,7%	63,0%	37,0%
Gentamicin	100,0%	0,0%	100,0%	0,0%
Imipenem	100,0%	0,0%	100,0%	0,0%
Penicillin	0,0%	100,0%	0,0%	100,0%
Doxiciclin	75,5%	24,5%	71,7%	28,3%
Ceftiofur (3rd gen. Ceph.)	100,0%	0,0%	100,0%	0,0%
Marbofloxacin	98,1%	1,9%	100,0%	0,0%
Enrofloxacin	98,1%	1,9%	100,0%	0,0%
Chloramphenicol	94,3%	5,7%	97,8%	2,2%

In conclusion, this study demonstrates that Gram-negative bacteria commonly isolable in case of mastitis are susceptible to most antibiotics, included fluoroquinolons, sulfonamide and chloramphenicol. Reduced susceptibility to some beta-lactams in *E.coli* are avoided associating clavulanic acid, an inhibitor of beta-lactamases. *Serratia* spp. is an exception probably due to specific beta-lactamases produced, which are resistant to inhibitors. These findings confirm the importance of milk culture in mastitis cases to achieve an etiological diagnosis, that allows to choose treatment or culling for chronically infected animals. The aims of treatment procedures in farm animal should be avoiding abuse of antibiotic therapy and reducing selection of resistant strains. Periodical survey and detection of resistance phenomenon are recommended.

References

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